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THE REPRODUCTIVE CYCLE AND
THE PITUITARY GLAND IN THE
SNAKE, THAMNOPHIS RADIX

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

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RELATIONS BETWEEN THE REPRODUCTIVE CYCLE AND THE PITUITARY GLAND IN THE SNAKE *THAMNOPHIS RADIX*

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WHILE studies of the seasonal changes in the reproductive cycle of the cold-blooded vertebrates appear in an ever increasing number, few studies have appeared which correlate these changes in both sexes with variations in the hypophysis. The physiological interrelationship of the hypophysis with the gonads is well established in the vertebrates (see Marshall, 1936; Van Dyke, 1939; Severinghaus, 1939; and Moore, 1939, for summaries and bibliographies).

Cyclical changes in the hypophysis are best interpreted in relation to the behavior of organs which show marked seasonal variations, such as the gonads. Among the cold-blooded vertebrates, correlative changes have been reported in the fishes (Bock, 1928; Matthews, 1936), amphibians (Zahl, 1937; Aplington, 1942), and reptiles (Altland, 1939; Poris, 1941).

The male and female garter snakes chosen for this correlative study present many curious problems regarding pitui-

tary control. Marked changes in the male gonads occur later than those occurring in the female. The sperm are produced during the summer months, while the ovocytes mature earlier. The sperm are stored in the ductus deferens during the fall and winter and are the effectual male germ cells the following spring. Copulation is not coincidental with fertilization and may occur long before ovocytes are mature. The female is ovoviviparous, with an approximate period of gestation of 9 weeks, during, and for a period after which, corpora lutea are present in the ovary. The corpora lutea in snakes have been shown to protect pregnancy (Fraenkel *et al.*, 1940; Clausen, 1940). Differential growth of ovocytes is present. Similarly, the pituitary state of the snake in hibernation, when the body temperature is considerably lower and all activities are minimal, is of interest.

Moore (1939) has pointed out the reciprocal action of gonadal-pituitary relations in warm-blooded animals. It is not unlikely that similar relations exist in the reptiles. Snakes have been shown to possess three sex hormones—estrone, progesterone, and an androgen (Valle and Valle, 1943). The effects of hypophysectomy and gonadotropins on the gonads of reptiles have been described.

In this study an attempt has been made to correlate the normal seasonal reproductive and pituitary cyclical changes in both the male and female garter snake,

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with the view of establishing relationships and a basis for physiological experiments.

MATERIALS AND METHODS

Only adult garter snakes, *Thamnophis radix* (Baird and Girard), were used for the seasonal studies. The male garter snake (20–51 gm.) is usually smaller than the female (42–164 gm.) and can be recognized externally by a thicker and longer tail. The female has an abrupt narrowing of the tail dorsoventrally, immediately posterior to the cloacal aperture. This difference is due to the presence of inverted hemipenes in the cloacal area of the male. In the male a probe can be passed into the sac formed by an inverted hemipenis (Schaefer, 1934). Sexual dimorphism occurs in the tail of the garter snake (Burt, 1928; Ruthven, 1908).

The animals used in this study were collected in a limited area of several square miles at the northern city limits of Chicago during the years 1937–38 and 1940–42. In all instances autopsy was performed within 1–3 days after collection, in order to avoid any changes under laboratory conditions. Hibernating specimens were removed periodically from an outdoor, escape-proof, wire-mesh cage ($7 \times 4 \times 4\frac{1}{2}$ feet), filled with leaves, or from a small indoor cage kept in a dark, cold-room (40° F.). A side door into the outdoor cage kept in a window pit permitted easy removal of the snakes with a minimum of disturbance to the environment. Usually the snakes were aggregated in the central part of the leaf pile. The top of the cage was covered with an oilcloth, to prevent undue wetting of the leaves. The skin of the snakes was moist or wet during hibernation, and the animals exhibited very sluggish movement, even when the outside temperature was below zero. It is of interest

that snakes in the cold-room were often found in the water kept in the cage.

For the reproductive cycle 101 males and 111 females were used throughout a period of 2 years. The pituitaries were studied from 35 males and 67 females autopsied throughout the 1-year period, and distributed as shown in Table 4 (p. 318). A total of 36 experimental animals were kept in a greenhouse.

Weights and measurements of the gonads were taken on the fresh organs. Bouin's and Zenker's fixatives, the paraffin method, and Mallory's triple, Delafield's, and Heidenhain's hematoxylin stains were employed on the gonads. The pituitary glands, with a part of the brain and floor of the skull, were placed in Dawson's fixative for 4 hours, washed, and run up to 70 per cent alcohol and iodine, at which time the hardened pituitary was dissected out. They were stained lightly with eosin in order to locate the gland in paraffin. Serial sections were cut at 5μ and stained with Mallory's Azan. Cell counts were made under oil immersion and with a $10\times$ ocular, using a Zeiss ocular crossline micrometer (5 mm. square). With a Bausch and Lomb microscope this gave a field of $6,400 \mu^2$. For each animal all the cells in the field were counted in two sections of the pituitary, and the average used as the recorded count.

ANATOMY AND HISTOLOGY OF THE PITUITARY

A. ANATOMY

The hypophysis cerebri of an average mature garter snake gave maximum measurements of 780μ in length, 918μ in width, and 535μ in height in the prepared section, although pituitaries may be larger or smaller than this, depending on the size of the snake. It consists of the pars anterior, intermedia, and nervosa (Pl. I, Figs. 1–4). No evidence of a pars

tuberalis is found in the adult. The vascular connective tissue from the anterior tip of the pars anterior penetrates and radiates in the thickened floor of the infundibulum (Figs. 1 and 3). This is the pars terminalis, which serves to attach the pars anterior. The anatomy of snake pituitaries has been considered by Siler (1936) and Hartmann (1944) and reviewed for reptiles by Charipper (1937). These observations on the pituitary of *T. radix* are presented as additional material to that given by Siler (1936) for the same species. Observations on the pituitary of *Thamnophis sirtalis* by Hartmann (1944) agree with those of Siler. Morphological and cytological studies of lizard pituitaries have also been made (Poris and Charipper, 1938; Altland, 1939). Poris and Charipper (1938) find similar divisions of the hypophysis in the lizard *Anolis carolinensis* except that a pars terminalis is absent.

Unlike the symmetrically arranged gland in other reptiles, the hypophysis of the snake usually has the bulk of the ventrally situated pars anterior displaced to the right and the pars nervosa to the left. However, occasionally in dissection the reverse relations have been noted, as well as a completely ventral and almost bilaterally symmetrical arrangement.

The residual lumen of Rathke's pouch is not the cleft between the pars anterior and posterior, which has never been found to contain colloid, but may be represented in part by the almost obliterated space of the pars intermedia, which often contains a "colloid" (Figs. 2 and 4) and the epithelial cysts.

The pars intermedia invests the pars nervosa closely and penetrates between the separate finger-like posterior projections of the pars nervosa (Figs. 1-4). The intermedia surrounds the nervosa ventrally, laterally, and posteriorly and

thins out anteriorly on the dorsal surface (Fig. 2). It varies in thickness from a double layer of columnar cells to dense regions where this layer is folded or corded. The surrounding connective tissue, containing vascular channels, is carried in with the folds. No vascularity is ever present between the double layer of cells. The pars intermedia at its posterior end narrows into a "stalk," which continues into the pars anterior (Figs. 2 and 4), not unlike the situation in the lizard (Poris and Charipper, 1938). The pars anterior turns ventrally; laterally, it becomes larger and then extends anteriorly, where it attaches to the infundibular floor by the pars terminalis.

The pars nervosa is smaller in volume than the pars anterior, while the pars intermedia is the smallest. The infundibular cavity (Fig. 2) does not extend into the digitate processes of the nervosa, as seen in the lizard (Poris and Charipper, 1938). Not all digitate processes are of the same length or diameter (Figs. 1 and 2). Their number varies in different pituitaries (cf. Figs. 2 and 4). The infundibular cavity is continuous with that of the third ventricle.

B. EPITHELIAL CYSTS

Ciliated, partially ciliated, and non-ciliated cysts of the pars anterior of the snake pituitary are quite common. They occur singly in 17 out of 75 females and in 6 out of 35 males examined. Four of the females possess two cysts. Cyst sizes vary from $24 \times 20 \mu$ to $90 \times 65 \mu$. The epithelium is quite variable in the different cysts. It may contain combinations of ciliated cuboidal epithelium, acidophiles, small and giant ($16 \times 12 \mu$) basophiles, and stratified squamous epithelium. The cavity occasionally contains colloid, but usually it contains cellular debris in various stages of disorganization.

C. HISTOLOGY

The acidophiles, basophiles, and chromophobes of the pars anterior show zonation (Figs. 1 and 4). The acidophiles occupy the greater part of the anterior half of the gland and then become mixed with basophiles and chromophobes in a narrow transition zone (Fig. 3). The chromophobe region most often shows a corded arrangement of cells (Fig. 4). The acidophiles constitute the largest proportion of cells; basophiles, the least.

The acidophilic cells are of two types, which, for convenience, are referred to as "red" and "pink" acidophiles. Those in the acidophilic area are large ($12 \times 16 \mu$), very irregular in shape, and often massed so that cell membranes are indistinguishable, heavily granular, and deep-red staining (Pl. II, Figs. 5A and 5B). Those in the chromophobe area (Fig. 1) that appear to be derived from the chromophobes are smaller ($8 \times 8 \mu$) and more regular in shape (Fig. 6). The cytoplasm either appears as a ring around the 4-6 μ vesicular nucleus or as a mass bordering the sinusoid. The cytoplasm contains fine granules, which are light-pink staining. In *T. sirtalis* the latter have been designated as orange cells, since the fine granules stain yellow to orange (Hartmann, 1944).

It is readily apparent that acidophiles extend over a larger area of the pars anterior during the period of activity of the animal, whereas during hibernation the area is less extensive. The peculiar morphology of the pars anterior does not allow a more precise comparison of the extent of these areas. Similarly, their numbers are greater during the period of activity, as evidenced by the massing of cells, while during hibernation they are sparser and more individual.

Attempts to trace numerical changes in the acidophiles are made difficult by the massing of coarsely granular cells,

which obscure not only cell membranes but also nuclei.

Throughout the year, acidophiles containing coarse granules can be seen, whereas the finely granular ones appear only during the active period of the animal (cf. areas in Figs. 1 and 3). The coarse granules are either sparsely distributed in the cytoplasm (Fig. 5B) or, as in the majority, so dense that they coalesce, giving a finely vacuolated appearance to the cytoplasm (Fig. 5A). Such finely "vacuolated" cells may show pycnotic nuclei. In some cells a few fine vacuoles attain a diameter of 1 μ . Several acidophiles have been seen with larger vacuoles.

The basophiles present two distinct types of cells, probably not unrelated. Numerous small basophiles ($6 \times 8 \mu$) with deep-blue-staining granules, which may be so dense as to appear coalesced, are present (Fig. 7A). This imparts a fine vacuolar appearance to the cytoplasm. Larger vacuoles are also found in the basophiles. Nests of small basophiles may possess pycnotic nuclei (Fig. 7B).

Larger basophiles, fewer in number and ranging from 8×8 to $14 \times 14 \mu$ in size, possess a light-blue staining, sparsely granular cytoplasm and large 6 μ vesicular nuclei (Fig. 8). These show variability in staining—sometimes almost a colorless condition. Vacuolization, pycnotic nuclei, and apparent dispersion of the cytoplasm are described in both sexes in the cyclic studies.

Chromophobes are small and stain poorly; their cell membranes are indistinct; very little cytoplasm is present; and usually the cells are quite crowded, as evidenced by their closely packed 3 μ nuclei (Fig. 9). Those in the chromophobe area sometimes stain a light lavender, not unlike cells of the pars intermedia.

Vascularization is quite pronounced in

the pars anterior. Blood cells are present in the sinusoids of most pituitaries (Table 4).

The elongated cells of the pars intermedia are arranged linearly in a double row, with the nucleated end of the cell centrad and the cytoplasmic end peripherad, the latter bordering the vascularized surface. The peripherad surfaces bound the pars nervosa and pars anterior. In thicker regions of the pars intermedia the covering vascular connective tissue is carried inward by folding. No vascular channels are ever found between the centrad ends of cells. Where the pars intermedia continues into the pars anterior by a narrowed "stalk," the vascularity of the former continues into the sinusoids of the latter.

Oxyphilic and basophilic granules in the same cells of the pars intermedia, bordering the cleft and basophilic granules in those cells adjacent to the pars nervosa, have been described (Siler, 1936). When Mallory's Azan was used, the greatest variability in staining reaction of these cells was encountered throughout the year in both sexes. The cytoplasm usually stained lavender to a deeper purple, was acidophilic and occasionally basophilic, or stained differentially, as described by Siler.

Variously sized clear-orange-staining globules, present in the "residual" lumen of the majority of the animals throughout the year, may be degeneration products derived from the nuclei of the intermedia cells, which show similar staining reactions (Figs. 2 and 4). Normal nuclei are vesicular and large ($6 \times 4 \mu$). Vacuolization of the centrad ends of the cells is pronounced in some animals.

Occasionally cells appear to be free in the lumen, but they may be the sectioned ends of cells which have become isolated. However, one pituitary possessed several typical pars anterior

basophiles; another, several acidophiles in the "residual" lumen.

SEASONAL CHANGES IN THE ADULT MALE

The male garter snake exhibited marked seasonal changes in weight and volume of the testes, associated with a single complete wave of spermatogenic activity during the summer. For this study a total of 101 males for the years 1937-38 and 1941-42, distributed as shown in Table 1, were used. The right

TABLE 1
SEASONAL TESTIS VOLUME AND
WEIGHT CHANGES

MONTH	1937-38		1941-42			
	No. of Animals	Av. Vol.* (Cu. Mm.)	No. of Animals	Av. Vol. (Cu. Mm.)	No. of Animals	Av. Wt. (Mg.)
Apr.....	5	165	3	219	3	179
May.....	5	243	5	216	5	116
June.....	3	252	4	243	4	163
July.....	8	476	3	558	3	366
Aug.....	8	820	4	654	6	491
Sept.....	7	724	8	489	4	386
Oct.....	9	815	1	597	1	439
Nov.....	5	204			1	386
Dec.....	2	151	3	266		
Jan.....	1	175	2	146		
Feb.....	1	75	3	110	3	124
Mar.....	8	186			3	114
Apr.....			3	114	3	92
Total...	62		39		38	

* Calculated for the ellipsoidal testis from measurements on the fresh gland.

ellipsoidal testis of the snake is usually larger and is located more anteriorly in the body. The mesial surface of the testis is bordered by an inconspicuous epididymis.

A. MACROSCOPIC CHANGES IN THE TESTES

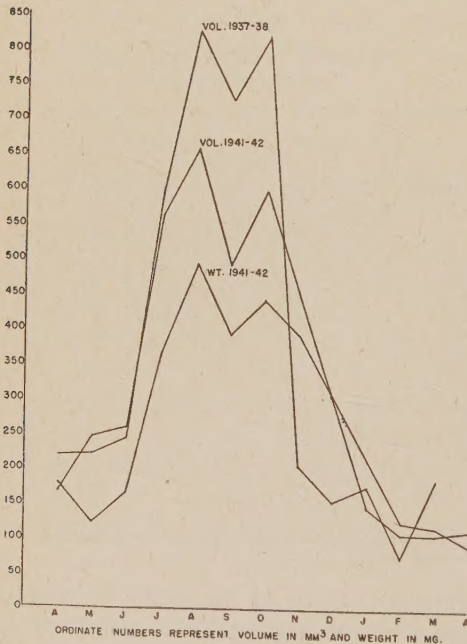
The volume of the testes for two seasons, as shown in Chart I, parallel each other closely. The difference in maximum volume of the 1937-38 series was attributed to larger animals used. The weight changes paralleled the volume changes. Testes of the snake were maximal in weight and volume from July to the end of October; minimal from De-

ember to May. In May they began increasing and in November decreasing. The maximum volume was 990 per cent and 500 per cent greater than the minimal volume, while the maximum weight was 430 per cent greater.

The slight but consistent temporary drop in weight and volume of the maximum-sized testes may be due to an emp-

CHART I

VOLUME AND WEIGHT OF TESTES OF *Thamnophis* THROUGHOUT THE YEAR



tying of the seminiferous tubules of the sperm, mature at that time, as well as of "fluid." The seasonal decrease in weight and volume occurred during the latter half of November, just as the animals entered hibernation, and was followed by a tapering-off during early hibernation. Testes were minimal in size when the snake emerged from hibernation in March.

In May the testes were grayish white and flaccid, becoming firmer, with an increase in size, by July. Increased

vascularity imparted a coral hue to the testes. When maximal in size, they were firm and white, with regression flaccidity increased and vascularity decreased.

Similar striking changes for other reptiles have been described, but only two species of turtles coincided in time relations: namely, maximal-sized testes in the summer, regression in fall, and minimal size in April and May, upon emergence from hibernation (Hansen, 1938; Risley, 1938).

In two species of male lizard, maximal volume and weight changes of seventeen times and three times that of the minimal have been given; however, the maximum appeared earlier in the season, during May and June (Blount, 1929; Turner, 1935).

In three other species of lizards, nearly maximum- or maximal-sized testes were attained late in the season (August and early fall), were maintained throughout hibernation, and regressed in June (Woodbury, 1938; Altland, 1941; Reynolds, 1943).

Other groups of lizards fall in late-spring or early-fall categories (Kehl, 1935). These gross seasonal changes, it will be seen later, are associated not only with spermatogenesis but also with the time of breeding and sperm storage.

B. SPERMATOGENESIS AND SPERM STORAGE

The spermatogenic cycle of the garter snake, as revealed by the study of the testes throughout the year, occurred as one complete wave during the period of activity of the animal. In hibernation no spermatogenic divisions take place.

The garter snakes in this area entered hibernation by November 1, although on unusually warm days an occasional snake would venture forth after this date as well as before March 1, about which time they began to emerge from hibernation.

Upon the snakes' entering hibernation the testes were not completely regressed in size. The seminiferous tubules were much collapsed, and the majority of the lumina were devoid of sperm. The germinal epithelium, however, still contained patches of elongated sperm heads and spermatids (Pl. III, Fig. 18). The picture during 4 months of hibernation showed a slow progressive destruction of these unevacuated and incompletely differentiated cells (Fig. 19). In the minimal-sized testes the tubules were one-half the diameter of the maximal-sized testes. In these reduced tubules the lumen was obliterated by debris derived from the germinal epithelium. This debris showed progressive signs of disintegration from pycnotic nuclei, multinucleated masses, and packets of sperm to a more homogeneous vacuolated granular mass. The germinal epithelium, much contracted by the shrinkage of the tubule, concentrated the nuclei of the Sertoli and the spermatogonial cells into a dense ring. There was no spermatogenic activity, only regressive changes, in the garter snake during hibernation.

Upon emergence from hibernation the lumina became clear. In April spermatogonia were dividing and primary spermatocytes appeared (Fig. 16). In June and July there was an intense spermatogenic proliferation, associated with the marked increase in size of the testis. By the end of July a few sperm tails were projecting into the lumen. Spermatids were abundant in the germinal epithelium. Only a few secondary spermatocytes were seen. Spermiogenesis was marked in August, and numerous sperm tails projected into the lumen (Fig. 17). The early stages of spermatogenesis were completed. Late in September sperm appeared free in the lumen of the seminiferous tubules, and they passed into the epididymis by the first part of October.

The passage of sperm was associated with the temporary decrease in the size of the testis. The remaining spermatids undergo spermiogenesis during the latter part of October. Rapid evacuation of most of the remaining sperm from the testis occurred just prior to entering hibernation.

Hansen (1938) found a correlation of spermatogenesis with seasonal size changes in the turtle *Terrapene carolina*, similar in time relations to that in the garter snake. In another turtle, *Sternotherus odoratus*, Risley (1938) described a single wave of spermatogenesis, with a further emptying of the seminiferous tubules in the spring. In the turtles the sperm were stored in the epididymis and used in fall or spring breeding.

Fall matings were reported for *T. radix* (Coues and Yarrow, 1878) and for *T. sirtalis* (Blanchard and Blanchard, 1941b; Blanchard, 1943). Trapido (1940) summarized evidence for fall mating in other species of snakes. As motile sperm can be secured from the ductus deferens of *T. radix* during hibernation, it is not unlikely that fall breeding is possible. Copulation, however, occurred most commonly after emergence from hibernation, from March until June, and may be repeated several times. Insemination may precede ovulation for varying periods, and consequently fertilization is delayed. The percentage of motile sperm in the ductus deferens markedly decreased in July and disappeared before the new sperm entered the ductus deferens. Therefore, only sperm produced during the preceding season are effective in fertilization.

Lizards with maximal-sized testes in the fall and throughout hibernation underwent an incomplete wave of spermatogenesis, with arrested activity during hibernation and the completion of spermiogenesis in the spring (Woodbury,

1938; Altland, 1941). Those lizards with minimal-sized testes during hibernation underwent spermatogenesis and achieved maximal size in the spring, utilizing the sperm in spring or early summer breeding (Blount, 1929; Turner, 1935; Clausen and Poris, 1937).

C. CHANGES IN THE INTERSTITIAL CELLS

Interstitial cells are present in the testes of the garter snake. They have been described for the lizards, snakes, and turtles (see Blount, 1929, and Herlant, 1933). The amount of interstitial tissue varied in the different groups. Herlant (1933) reported scattered little masses in the lizards and more voluminous masses in the ophidians and chelonians. Altland (1941) found no interstitial cells in *Sceloporus u. undulatus*. Mazzetti (1911) reported them as abundant in a hibernating snake. In the hibernating garter snake the interstitial cells occurred in compact nests in the intertubular spaces. Relative to the regressed state of the seminiferous tubules, they appeared abundant, whereas in maximal-sized testes the cells were dispersed and appeared relatively less voluminous.

The interstitial cells in the lizards were best developed when the secondary sex characters were at their maximal activity in May (Herlant, 1933; Regamey, 1935). In the adder the interstitial cells did not appear to vary (Herlant, 1933), nor did Risley (1938) observe any changes in the musk turtle. Blount's (1929) quantitative study of seasonal variation in number and morphology of interstitial cells and volume of interstitial tissue demonstrated an increase in volume and a decrease in number concomitant with spermatogenic activity.

Seasonal observations on the interstitial cells of the garter snake revealed

evidences of secretory activity and variation in size and staining.

In the spring the cells were large ($16 \times 18 \mu$) and contained eccentrically placed, spherical, vesicular nuclei (8μ); they occurred in compact nests; their cell membranes were distinct and presented a polyhedral pattern (Fig. 16). The cytoplasm was light-staining; the fine granules formed a delicate alveolar protoplasmic pattern. Only an occasional cell showed an irregularly shaped, smaller (6μ), denser nucleus and vacuolated cytoplasm.

In July and August the cells were more dispersed, although a few nests still appeared. Some cells, with irregular, darker nuclei, had very light vacuolated cytoplasm and, because of the indistinct cell membranes, appeared to be continuous with a similar light-staining intertubular substance showing vacuolation. It appeared that these cells were actively secreting (Fig. 17). Smaller cells ($10 \times 12 \mu$), with a distinct cell membrane, denser cytoplasm, and an eccentrically placed (6μ) nucleus, and the spherical (8μ) cells with 5μ vesicular nuclei may be either postsecretory or new cells.

In October, just before entering hibernation, the large interstitial cells were again crowded in nests. No intertubular substance was present. The most striking condition was the presence of enormous interstitial cells ($14 \times 26 \mu$) with 8μ , vesicular nuclei. The cytoplasm was homogeneous or vacuolated (arrow, Fig. 18). Other cells averaged $10 \times 12 \mu$ and possessed a vesicular nucleus.

During hibernation many of the large cells ($14 \times 16 \mu$) were vacuolated but had a distinct cell membrane and an irregular small nucleus (arrow, Fig. 19). The remaining cells measured $10 \times 12 \mu$, and possessed a 6μ nucleus. The cytoplasm in the latter was denser. In some in-

stances cell membranes were indistinct where the cells occurred in groups.

In the garter snake large interstitial cells are present in the spring; during the summer they are small and are dispersed in an intertubular substance; in the late fall they attain their greatest size; in hibernation the large cells are vacuolated, while the smaller cells occur in groups and possess an indistinct cell membrane and an irregular nucleus.

The lizard *Phrynosoma* had different time relations in its spermatogenic activity and breeding. Breeding immediately followed the production of sperm. Blount (1929) correlated the greatest size of the interstitial cells with the height of the breeding season.

Breeding in the garter snake does not immediately follow the production of sperm, as in the lizard *Phrynosoma*. However, there is a possibility of fall mating in the garter snake, at which time interstitial cells are large, some attaining enormous size. Interstitial cells in the garter snake are also large in the spring, when breeding is pronounced.

D. CYCLICAL CHANGES IN THE PITUITARY OF THE MALE

Thirty-five adult males, distributed throughout the year 1941-42 (Table 4), were used for the study of the seasonal cell changes in the pituitary. Although the pituitaries were from animals sacrificed weekly, the counts represented in Table 4 are monthly averages. As described earlier, two counts of acidophiles, basophiles, and chromophobes were made on each animal.

Certain inherent difficulties are present in making a quantitative analysis of the snake pituitary. Because of the peculiar asymmetrical, teardrop shape of the pars anterior, it is not possible to make a strip count which gives the relative proportions of the areas occupied by chro-

mophiles and chromophobes. However, qualitative observations have been made on serial sections, and these will be added. A second factor that is readily apparent, when using a given area, is the variation caused by the distended or collapsed state of the sinusoids and by differences in cell size. Both large and small, acidophiles and basophiles, are combined in the counts. A separation of each chromophile into two types, on the basis of size, would imply that the cells are not related. Also, cells of intermediate size would be difficult to place. Attention is also called to the fact that, whereas a second peak in number of basophiles is present during hibernation, these cells are predominantly small in size; their increased number does not mean that physiological activity is present. Because the areas selected are in a transition zone where the basophiles are present, the counts of acidophiles and chromophobes are not a true indication of their actual abundance. Qualitative changes of all the cells will be considered; numerical changes, only for the basophiles.

In the pars anterior two types of acidophiles were described earlier. The coarsely granular acidophiles are present throughout the year in the acidophilic zone. They are abundant and closely packed in this zone during the spring, summer, and fall, and sparsely distributed during hibernation. Throughout the year the majority of these acidophiles show deep-red-staining granules, so packed as to appear coalesced and "vacuolated" (Fig. 5A); the remaining ones have fewer deep-red granules, which are sparsely distributed in the cytoplasm and are seen chiefly in the transition zone from spring until fall (Fig. 5B). The latter acidophiles may be in a process of degranulation. Orange-staining nuclei are present in some

acidophiles during the summer and may be indicative of an altered physiological state.

The finely granular, pink-staining acidophiles are most abundant and pronounced in the chromophobe area during the spring; they become fainter in the summer and fall, decreasing in abundance until in hibernation the chromophobe area is almost pure chromophobes. These finely granular acidophiles are small in size; they contain little cytoplasm; and the nuclei are centrally placed or acentric and stand out clearly. They are not unlike the chromophobes in morphology and are located in a zone which is chromophobic during a part of the year. Their origin from the latter appears certain. Between the coarse acidophiles, which are sparse during hibernation, are chromophobes. These chromophobes must be the source of coarse acidophiles that fill this region during the active period. The two types of acidophiles do not appear to be stages of secretory activity of the same cell but do appear to arise independently from chromophobes. However, in *T. sirtalis*, Hartmann (1944) describes carmine (red) and orange (pink) acidophiles as morphologically different and states that some carmine cells arise directly from chromophobes, whereas others are derived by transformation from orange cells.

The coarsely granular acidophiles are least abundant in hibernation, and the finely granular acidophiles are most pronounced in spring. Both types of acidophiles are active in producing secretions when climatic and food conditions are optimal for growth (see discussion for association of acidophiles with growth hormone on p. 322).

The basophiles are generally considered in the literature to be the source of gonadotropic substances (Severinghaus, 1939). Seasonal changes in the number of

basophiles, as represented in Chart II, reveal two peaks of abundance. The higher and more significant peak occurred during the period of testicular activity, while the second, lower peak occurred during hibernation, when testicular activity was absent.

In the spring the large light-blue basophiles are most abundant. They show the most pronounced cytological change—a change which is undoubtedly associated with the elaboration of gonadotropic substances. The granules are fine and are dispersed in the cytoplasm. The staining reaction of the cells decreases until they are almost colorless. Cell membranes become progressively less distinct. Vacuolization appears in the cytoplasm, and many nuclei become orange in color and irregular in shape. Only a few small deep-blue basophiles are present at this time.

In *T. sirtalis* during periods of decrease in basophile percentage many of the cells stain less blue than the rest, and pycnosis of the nucleus may or may not be present. The cells contain extremely fine blue granules in a colorless cytoplasm with a quite centrally situated nucleus and a prominent nuclear membrane (Hartmann, 1944).

The variability in staining response of the basophiles encountered by three workers on garter-snake pituitaries, in spite of attempts at a uniform technique throughout the year, is sufficient reason to believe that the condition of this cell varies.

In the summer, only a few light-blue basophiles, with either scattered granules or vacuoles, are present. Small bodies, 3 μ in size, which occur in nests, appear to be the end stage of light-blue, vacuolated basophiles which have given up their secretion (Fig. 7B). Only a faint-blue, irregular cytoplasmic border surrounds a much shriveled nucleus which

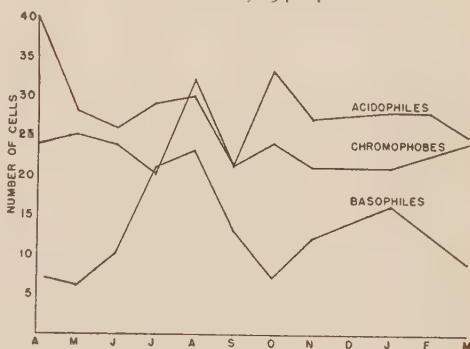
may be temporarily pycnotic. Whether these bodies degenerate, form chromophobes, or regenerate new basophiles is difficult to ascertain. The small deep-blue basophiles are appearing at this same time in ever increasing numbers. Their nuclei are quite dense and spherical and in some cases they are orange and irregular. The coarse granules of the cytoplasm are closely packed and appear coalesced. This gives a finely "vacuolar" appearance to the cytoplasm (Fig. 7A). Occasionally, larger vacuoles do appear in these cells. When judged by their shape, size, and amount of cytoplasm, the deep-blue basophiles seem to be derived from chromophobes, although the nuclear condition might lead one to believe that they arise from the expended basophiles by regeneration. That both phenomena may occur in the pituitary has been shown by the study of mitotic activity, which gives rise to a new supply of chromophobes (Hunt, 1942), the degeneration of cells (Kirkman, 1937), and regeneration (suggested by Aplington, 1942).

In the fall the light basophiles are no longer apparent; only small deep-blue cells with a homogeneous granular cytoplasm are present. Nuclei are clear and vesicular. These cells gradually increase in size and in number. They are the dominant basophile of the hibernating snake. A few, slightly larger basophiles, with a homogeneous light-blue cytoplasm which are present during hibernation, apparently arose from the deep-blue cells and will become the large light-blue basophiles seen in the spring pituitary.

Of the two seasonal peaks in basophile number represented in Chart II, only the summer peak can be correlated with maximum gonadotropic activity. At the time of emergence from hibernation and during the spring there was a progressive decrease in the number of basophiles.

Cytologically, the basophiles exhibited marked secretory activity in the spring, when testicular activity was stimulated. During the height of testicular activity in the summer, small deep-blue basophiles increased in number and in extent. Whether the fine "vacuoles" and occasional larger vacuole in the small basophiles represent activity is not certain. Light-blue basophiles were present during the summer but decreased toward the end of the spermatogenic period.

CHART II
SEASONAL CELLULAR CHANGES IN THE MALE
PITUITARY, 1941-42



When the testes attained their maximal size in the summer, the basophiles were three times as numerous as during the spring. Physiologically, the pituitary is active gonadotropically during this period, as demonstrated by experiments in Section E, below. The decrease in basophile number and extent in September and October was associated with a rapid decline of spermatogenic proliferation and a temporary decrease in the size of the testes. Along with the decline in number of basophiles, sperm appeared free in the lumen of the seminiferous tubules in September and in the ductus deferens in October. It may be that the release of sperm from the testis is induced by gonadotropic substances, just as ovulation may be induced in the female.

When the basophiles are minimal in number in late October (the testes have completed a secondary wave of spermioteleosis, and interstitial cells have attained an enormous size), a rapid decrease in size of the testes occurs. As climatic and food conditions become less favorable, the animals enter hibernation. The basophile number slowly increases upon entering hibernation, does not attain the height of the first peak, and does not show the cytological changes associated with secretion. Spermatogenic activity is absent in the testis, and interstitial cells are vacuolated. Cellular activities in the hibernating animal are held minimal by the lowered temperature. At 5° C., Mellish and Meyer (1937) showed that the stimulating effects of mammalian gonadotropins in a lizard could be abolished. Aplington (1942) showed that in the chromophiles of the *Necturus* winter hypophysis the Golgi apparatus was closely applied to the nucleus, which is interpreted as a non-secretory phase.

Differential counts of chromophiles and chromophobes in 22 male garter snakes (*T. sirtalis*) revealed two peaks in basophile numbers also (Hartmann, 1944). These peaks occur in April and September. The reproductive cycle of this species, distributed around Ithaca, New York, is not known; but Hartmann indicates that the increased basophile number may possibly be correlated with increased size of the testis and with spermatogenic activity at these periods.

E. HYPOPHYSECTOMY AND REPLACEMENT EFFECTS ON THE TESTES

Twenty garter snakes were hypophysectomized on June 30 and July 9 (Table 2), to determine to what extent the pituitary gland, which showed increasing numbers of deep-blue basophiles and relatively few light-blue basophiles,

was secreting a gonadotropic substance. At this time the testes were markedly increasing in size (Chart I), and spermatogenesis was quite intense.

The snakes were anesthetized by a subcutaneous injection of sodium amytal, supplemented by ether. The actual operation is relatively simple because the easily located basisphenoid projection containing the pituitary is approached directly and is not obscured by the palatine bones. Bleeding was minimal or absent; healing rapid; and infection absent. Hellbaum (1936) presents details of the operation as described by Schaefer (unpublished).

Pituitaries have been successfully removed in other cold-blooded vertebrates. Matthews (1939) found that *Fundulus* males that were hypophysectomized in fall and kept until the next breeding season (spring) failed to show typical testis enlargement, while males hypophysectomized in March or April, when testes are enlarged, showed a reduction in size below that of the controls, and, in addition, spermatogenesis was affected. Similar regressive changes have been reported in another teleost, *Gobius pagnellus* (Vivien, 1938, 1939). Marked retrogression in size and histological disorganization of the testes in *Amblystoma tigrinum* (Burns and Buyse, 1932) and marked involution in *Triton cristatus* (Wořonzowa and Blacher, 1930) have been reported. Hypophysectomized cold-blooded vertebrates showed marked changes in the testes and a gonad relationship similar to that established in higher vertebrates.

Male garter snakes hypophysectomized on July 9 and autopsied 9 and 16 days later (controls for replacement experiments) showed only a slight difference in weight of the testes, when compared with the normal cycle (Chart I). Histologically, after 9 days the germinal epithelium un-

derwent a marked sloughing of cells, leaving the epithelium as a thin ring of cells. The sloughed cells completely filled the lumen of the seminiferous tubules. A few tubules showed pycnotic cells and macrophages (Fig. 10). Interstitial cells are clumped and small. Sixteen days after hypophysectomy the tubules are much shrunken. Pycnosis, dissolution, and vacuolization are pronounced (Fig. 12). Interstitial cells are small and compacted, and they possess

color and flaccid. Histologically, the solid seminiferous tubules ($63\ \mu$ in diameter) showed a germinal epithelium disrupted, vacuolated, and containing cellular debris and pycnotic nuclei, or a single row of spermatogonia and Sertoli cells (Fig. 14). No active spermatogenic divisions were present. Interstitial cells occurred grouped either with irregular nuclei containing dense chromatin, with indistinct cell membranes and little cytoplasm, or individually (measuring $10\times$

TABLE 2
HYPOPHYSECTOMY AND REPLACEMENT EFFECTS ON THE TESTES

No. OF ANIMALS	TREATMENT (1942)			AVERAGE ANIMAL WEIGHT (Gm.)	TESTES	
	Hypophysectomy	Autopsy	Treatment		Average Weight (Mg.)	Average Volume (Cu. Mm.)
3.....	June 30	Aug. 12	None	24	40	46
3.....	June 30	Sept. 3	None	28	36	39.9
3.....	Intact	Sept. 3	None (control)	30	393	406
5.....	July 9	July 18	10 male pituitary homeo-implants each	30.6	380	
2.....	July 9	July 18	Control	28.5	185	
5.....	July 9	July 25	Total each 350 I.U. Gonadin (equine gonadotropin)	28	421	
2.....	July 9	July 25	Control	36	227	

irregular nuclei and vacuolated cytoplasm.

Hypophysectomized males showed over a prolonged period (operated on June 30 and autopsied 44 and 66 days later) a marked reduction in the testis weight and volume (Table 2). The normal control testis size was ten times greater than that of the experimental animal. When the atrophy during this period is compared with the size changes in the normal seasonal cycle (Chart I), a 50 per cent reduction below that which occurs during hibernation (testes normally minimal in size) is found.

Macroscopically, the testes of the hypophysectomized snakes were tan in

$12\ \mu$; nuclei, $3-5\ \mu$) with vacuolated cytoplasm, densely chromatic, irregularly shaped nuclei, and an occasional vesicular nucleus. No sperm were present in the ductus deferens.

The coral-colored testes of the normal controls, in marked contrast to the experimentals, showed large distended lumina in the seminiferous tubules ($150\ \mu$) and a thick germinal epithelium with abundant normal divisions in the later stages of spermatogenesis (Fig. 15). The spermatids and elongating sperm heads were most numerous. Sperm were present and free in the lumen. The testis of the controls corresponded in size and phase of spermatogenesis to that seen

in the normal cycle. The intertubular connective tissue showed a pronounced vascularization and wide areas of a finely granular, light-staining colloid in which the interstitial cells were mostly scattered (Fig. 11). The interstitial cells were large ($12 \times 16 \mu$), with eccentrically placed vesicular nuclei and finely alveolar, light-staining cytoplasm. Cells measuring $12 \times 14 \mu$ had a densely chromatic nucleus and a heavily vacuolated, light-staining cytoplasm which was not distinguishable from the surrounding vacuolated substance of the intertubular space. Smaller cells ($8 \times 10 \mu$) had a distinct cell membrane. An occasional spherical interstitial cell (7μ) with dense, heavier-staining cytoplasm contained an irregularly shaped densely chromatic nucleus. It appeared that the interstitial cells in phases of secretory activity contributed to the abundant colloidal material surrounding the cells. The interstitial cells of the controls were like those in the normal cycle on September 3. When the interstitial cells of the hypophysectomized animals are contrasted with those of the controls, far less evidence for secretory activity was found to be present in the former. The cells were smaller in size, contained less cytoplasm, and possessed denser, irregular nuclei.

In hypophysectomized *T. radix* and *T. sirtalis*, Schaefer (1933) observed "small and flaccid testes with degenerating spermatocytes in the atretic seminiferous tubules and the size of the interstitial cells less than normal." His observations were made during the winter months, when the testes are normally either regressing or minimal in size and inactive.

Removal of the pituitary gland in July (at a stage when small deep-blue basophiles are increasing in number and degranulating light-blue basophiles are

present in relatively fewer numbers) caused the failure of testicular activity. Animals hypophysectomized over a prolonged period during the summer showed atrophy and aplasia of the testes. The withering of the gland following hypophysectomy to a level below that which occurs in hibernation would indicate that even in the reduced inactive testes of hibernating animals there is hypophyseal influence on the testes.

July male pituitaries were used for implantation to determine their gonadotropic potency. Fresh glands were macerated in distilled water and injected intraperitoneally. Each of five hypophysectomized males received a total of ten glands in four injections. The weight of the testis after 9 days was twice that of the controls (Table 2). Compared with the normal cyclical changes (Chart I), when the testes rapidly increase in size, the homeo-implants produced not only the normal 50 per cent increase in weight during this period but also an added stimulation of 50 per cent. Spermatogenesis was abundant and normal, with differentiation of sperm (Fig. 11). Interstitial cells were mostly intermediate in size, and the cytoplasm dense. Only a few larger cells ($17 \times 16 \mu$) were present (cf. with Fig. 10, described above). The gonadotropic potency of July male pituitaries was sufficient not only to maintain testicular activity and produce the normal increase in testis weight during this period but also, in the amount administered, to stimulate a further increase. Schaefer (1933), in winter snakes, reported that subcutaneous implantations of a single hypophysis every other day for a period of 30 days elicited germ-cell production, enlarged tubules, and increased size of interstitial cells.

Another group of five hypophysectomized males was daily injected intraperitoneally with equine gonadotro-

pin (Table 2). Each received a total of 350 I.U. and was autopsied 16 days later. The weight of the testis was practically doubled, and spermatogenesis was abundant. Interstitial cells were all greatly enlarged, and many of them were vacuolated. (Cf. Fig. 13 with Fig. 12 [hypophysectomized control described above] and Fig. 11.) This stimulation, like the homeo-implant stimulation, was similarly in excess of that which occurs normally in the seasonal cycle.

The effectiveness of mammalian pituitary extracts in stimulating reptilian gonads has been demonstrated. This is considered later in the discussion.

only slightly higher in number than that in the control, extended over a wider area. When compared with the normal basophile cycle, the number was found to be low at the time of castration. It is significant that, whether testes are present or absent, the basophiles show a three-fold increase during this phase of the seasonal reproductive cycle. This same phenomenon may account for the slight increase in percentage of basophiles reported by Poris (1941) for the castrate male lizard *Anolis*. In *Triturus viridescens* 3½-month castrates showed an increase in basophiles two to three times the normal (Copeland, 1943).

TABLE 3
CASTRATION EFFECT ON THE PITUITARY OF THE MALE

No. of Animals	Treatment	Av. Weight of Animals (Gm.)	Av. Weight of Testes upon Removal (Mg.)	Av. Pituitary Cell Counts (Acidophiles-Basophiles-Chromophobes-Erythrocytes)
2.....	Castrated 5/2/42; autopsy, 8/12/42	21	70	27-20-23-3
2.....	Castrated 5/2/42; autopsy, 9/4/42	35	97	19-25-23-9
3.....	Normal control; autopsy, 9/4/42	30	427	23-20-19-9

F. EFFECT OF CASTRATION ON THE PITUITARY IN THE ADULT MALE

Castration of four males was performed on May 2, 1942, at a time when the basophile count in the normal cycle was at its lowest (Chart II). Two animals were autopsied after 103 days; and two others after 124 days, when three controls were sacrificed. Not only did the weights of the testes of the castrates at the time of removal (av., 83 mg.) and of those of the normal controls at the time of autopsy (av., 427 mg.) (Table 3) agree with the weights of the testes in the normal cycle, but the histological picture at those phases of the cycle was also the same.

One hundred and twenty-four days after castration the basophiles, although

Cytologically, pituitaries of castrates and controls showed a predominance of small deep-blue basophiles with coarsely granular cytoplasm, either dense, appearing "vacuolated," or scattered. The basophiles of the castrate extended over a greater area and contained a larger percentage of orange nuclei (cf. Figs. 20 and 21). Only a few light-blue basophiles (described in the normal cycle) contained scattered coarse granules, and occasionally small vacuoles were present. Small bodies described in the normal cycle and believed to be expended basophiles were present also in the castrate. Coarsely granular and finely granular acidophiles were present in both castrate and control; however, the finely granular pink acidophiles were still quite numerous and

distinct in the castrate, while in the controls only a few faint ones were present. No acidophilic mass in the basophiles, as reported in 4½-month castrate lizards (Poris, 1941), was observed in the snake.

SEASONAL CHANGES IN THE ADULT FEMALE

The reproductive cycle of the female, in contrast to that of the male, involved production of mature eggs and their utilization during the same season. This ovoviviparous species gave birth to young after a gestation period of 9 weeks.

The elongated saclike ovaries varied in length from 2.5 to 10.5 cm. The right ovary was always more anterior than the left and 0.8–1.6 cm. longer. The ovocytes were linearly arranged, giving the gonad a beaded appearance. Ovocytes of several sizes were distinguished; smaller ones were translucent, and larger ones opaque-yellow. As yolk accumulated, the ovocytes enlarged. At their maximum size the ovocytes measured 1.6×1.1 cm. and were orange-yellow in color.

Upon ovulation a corpus luteum formed, persisted throughout pregnancy, and regressed very slowly after parturition. The luteal tissue arose from the granulosa layer (Rahn, 1939) and has been described for other reptiles (see Fraenkel *et al.*, 1940; Boyd, 1940). Post-pregnancy females could be identified definitely up to and during early hibernation by the still vascularized, unregressed oviducts, as well as by the corpora lutea. In the spring the corpora lutea had regressed to small orange specks and were not distinguishable from those discernible from former pregnancies. The number of corpora lutea scars was not suitable for certain identification of more than one pregnancy, since the garter snake has a variable number of young per brood. Mature virgin females could be readily identified not only by the ab-

sence of corpora lutea scars but also by the presence of an imperforate membrane, where the paired Müllerian ducts join the urodaeum.

A. COPULATION

The female is inseminated in spring, prior to ovulation. One pair was observed in the act of copulation in the field on May 1. The pair remained in coitus ½ hour after being collected. In the field, on April 27, and in the laboratory, on April 29, courtship was observed. An account of courtship behavior and mating in the garter snake has been described by Davis (1936) and the Blanchards (1942); in other species, by Noble (1937). Sperm have been found in the oviducts of spring females but not in hibernating females. (See the section on the male, Part B, for a discussion of fall copulation.) The sperm are held until fertilization, which occurs in late May or early June, at the time of ovulation. Rahn (1940) reported motile sperm in the washings of the oviducts of *T. s. sirtalis* 1 month before fertilization, which occurs during June and early July. In the copperheads, sperm live 11 days or longer in the female (Gloyd, 1934). Isolated oviparous species of snakes have remarkable records of sperm viability in their reproductive tract, as evidenced by their laying of successive clutches of fertile eggs. Sperm in the female are capable of fertilization in the African night adder over a period of 81 days (Woodward, 1933), in the Malayan water snake over a period of 1½ years (Kopstein, 1938), and in a Central American leptodeiran over a period of at least 5 years (Haines, 1940).

B. PERIOD OF GESTATION

The period of gestation in the garter snake was approximately 9 weeks. In 1941 the earliest ovulation was observed on May 31, and the latest date of no

ovulation was on June 21 (Chart III). Whether the phenomenon occurs in the early or late parts of this period is probably dependent on the temperature (Blanchard and Blanchard, 1941a). The earliest parturition occurred on August 2, and the last female to be pregnant was observed on August 23. Most of the females had given birth, however, the first week in August. There was a 3 weeks' variation at either end of the period of gestation. Assuming that the earliest ovulation produces the earliest brood, the period of gestation would be 9 weeks. Variations in gestation periods for other species of snakes have been found (refer to Clausen, 1936, for summary), as well as variations in the incubation period of oviparous snakes. As shown for this ovoviviparous species, the gestation period cannot be dated from the time of copulation. The unduly long gestation periods reported need re-examination.

C. EFFECT OF HIBERNATING CONDITIONS ON THE GESTATION PERIOD

Six females in the latter half of their gestation period were placed under hibernating conditions (dark, cold-room, 40° F.) on July 23, 1942, and removed to room temperature after 70 days, to determine the effect of temperature on the period development of the embryo. None gave birth to fully developed young during this period. One died upon removal, October 21; two on November 12; a fourth on November 23; and a fifth on April 23, 1943. Upon autopsy the retained young were found to be in various stages of resorption and showed that development had been arrested. Half the amount of yolk present in embryos in the latter half of their developmental period was still present. The sixth pregnant female aborted (five times between January 30 and March 15, 1943) a total of 9 dehydrated, dead, half-grown em-

bryos and was still alive on April 26. The embryos undoubtedly died during the period of hibernation, as evidenced by their degree of development.

D. NUMBER OF YOUNG IN THE GARTER SNAKE AND CORPORA LUTEA

The number of developing young in the garter snake was quite variable, ranging in 1937-38 from 9 to 22, in seven females, averaging 15.4 young per female. In 1941-42 the developing young ranged from 6 to 26 in twenty-six females, averaging 12.4 young per female. In a large (37 inches) *T. s. sirtalis* a brood of 73 young was reported by Wallace (1938). Usually the number of young is much fewer. Blanchard and Blanchard (1942) found in their extensive studies on *T. s. sirtalis* that the number of young varied from 6 (the first brood of a 3-year-old female) to 51. In eight gravid *T. s. sirtalis*, Burt (1928) found a range of from 12 to 32 young, or an average number of 16.9 young; and in five *Thamnophis sauritans sauritans*, a range of from 4 to 8, or an average number of 6 young. For the latter subspecies Ruthven (1908) found 12 young. Kopstein (1938) reported similar ranges in seven other ovoviviparous species of snakes.

An analysis of the distribution of young in the oviducts showed an average of 8 in the right and 4.4 in the shorter left. In these same females the corpora lutea average 8.4 in the right ovary and 6.1 in the left ovary, which indicated that not all ovulated ovocytes developed. Corroboration was found in several instances of undeveloped, dehydrated ova in the oviducts. An analysis as to whether ovulated ovocytes entered their respective oviducts revealed, in four cases, that migration occurred from the left side, anteriorly to the right oviduct. Similar observations were made in the turtle (Agassiz, 1857).

Where fewer ova than corpora lutea occurred, there is the possibility that the ova were lost. That contractions in either direction, causing expulsion of the contents of the oviducts, can occur during gestation is believed to be indicated in the following two cases. A coelomic pregnancy, autopsied 2 months after birth should have occurred, showed young in stages of resorption. This may have been caused by a reversed contraction sometime during development. Another female undoubtedly aborted her young during mid-pregnancy, as evidenced by heavily vascularized, collapsed oviducts, well-developed corpora lutea in the ovaries, and a high basophile count.

E. SEASONAL CHANGES IN THE OVARIAN CYCLE

The ovaries and reproductive tract showed seasonal changes (Cieslak, 1938). The area of a median longitudinal section of the ovocytes offered a convenient index for size changes. These changes in 111 females for the years 1937-38 and 1941-42 are represented by the average mean curves of Chart III.

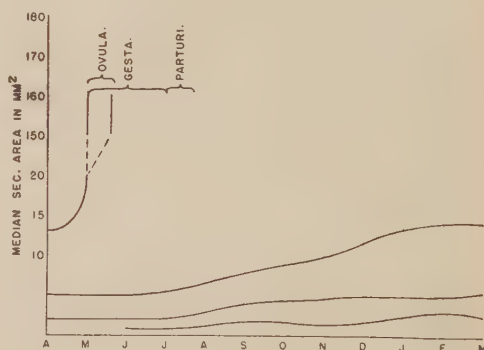
Three distinguishable size groups of ovocytes were present in the mature female, which could be measured macroscopically. The largest size group in May rapidly increased in size from 13 sq. mm. to 140-226 sq. mm. The maximal size variation appeared to be associated with the total number of ovocytes. Where large numbers were present, the mature yolk-laden ova were smaller. Ovulation of this group occurred on the last day in May and during the first part of June. Corpora lutea developed and were large during pregnancy, regressing slowly after parturition. It is believed that the tiny orange specks that are present during hibernation in mature females having their oviducal membranes ruptured represent corpus luteum scars. From the

numbers of these in some females it appeared that they had accumulated from former pregnancies. Large virgin females with unruptured membranes never showed these corpus luteum scars.

During the growth of the first group of ovocytes there was little change in the size of the second and third groups. At the end of June, after the females had ovulated and most of them were in mid-pregnancy, a new group of tiny ovocytes, slightly less than 1 sq. mm., made its appearance, restoring the three size groups

CHART III

CHANGES IN THE THREE GROUPS OF OVOCYTES
OF 111 *Thamnophis* THROUGHOUT
1937-38 AND 1941-42



in the ovary. There was a very slight growth of the new first and second groups in July and August. During the early part of September all three groups grew slowly; and they continued to grow through the first half of the hibernation period, at which time growth leveled off, so that, upon emergence from hibernation the following year, the three groups attained the same size categories seen the previous year. The number of ovocytes in each of these three categories showed the same variation in numbers as the broods.

In some animals, such as the mouse, the germinal epithelium becomes active at each normal oestrus period, forming

cyclic proliferations of germ cells and follicle cells (Allen, 1923). Woodbury (1938) described part growth of lizard ova in fall, no change in winter, and rapid enlargement in spring, when ovulation occurred. Four well-marked sets of different-sized ovocytes have been described in the ovary of *Chrysemys picta* (Agassiz, 1857). In the fish *Cottus bairdii* a portion of the ovocytes formed during the first season matures for the first spawning, which takes place at the age of 2 years. The remainder form a reserve supply, which is increased each year by ovocytes from dormant oögonia (Hann, 1927).

F. SEASONAL CHANGES IN THE FEMALE PITUITARY

In considering the cyclical pituitary changes in the female, it was necessary to consider the sixty-seven mature females in four categories: (1) nonpregnant females (April-June), (2) pregnant females, (3) postpregnant females, and (4) mature virgin females. The determination of these categories was quite definite, according to criteria described earlier, in Part E, on the ovarian cycle. The females were distributed throughout the year, as shown in Table 4. Cell counts of the pituitary in Table 4, plotted in Chart IV, necessarily overlap, because of the 3-week leeway in the period of gestation.

Cyclical changes in the coarsely granular acidophiles, present throughout the year, were similar to those in the male. These were large, irregular in shape, and "vacuolated" throughout the year. During hibernation they were less concentrated in the acidophile zone. In the spring the granules in some of these cells were scattered, and in the fall this condition was more pronounced. The cells with scattered granules may be degranulating and returning to a chromophobe state, which would then account for their

sparser distribution during hibernation. The finely granular pink acidophiles undergo a more marked seasonal change. In the nonpregnant females and the pregnant females they are abundant in the chromophobe area. In the postpregnant females in hibernation they are absent.

The dominant basophiles in the nonpregnant females in April and June are of the large light-blue type. The cytoplasm is sparsely granular and contains numerous tiny vacuoles or a few larger ones (Fig. 8). Cell membranes are indistinct, and the cytoplasm becomes almost colorless. Those cells lining the sinusoids appear disrupted, as if discharging the light-blue colloid in the sinusoid with which they are continuous. Orange nuclei are also seen. The light-blue basophiles showing cytological evidence of secretion occur also in pregnant females but appear fewer in proportion to the deep-blue basophiles in the latter half of pregnancy. In postpregnant females in the fall they are still present and active but fewer in number. In early hibernation a few smaller light-blue basophiles with scattered granules are preparatory for the more numerous, larger light-blue basophiles with tiny vacuoles, which appear in late hibernation.

The deep-blue basophiles are not apparent in the spring but appear in early pregnancy and become very numerous in late pregnancy. They are present in the postpregnant females in fall, predominate in early hibernation, form two-thirds of the cells in late hibernation, and give rise to the light-blue basophiles in spring. During pregnancy and postpregnancy in the fall, the presence of coarse granules in these small deep-blue cells (which appear to be coalesced, giving a "vacuolated" appearance), occasional larger vacuoles, and variable amounts

TABLE 4
DISTRIBUTION OF ANIMALS AND MONTHLY AVERAGE OF PITUITARY CELL COUNTS

MONTH (1941-42)	NO. OF FEMALES (ACIDOPHILES-BASOPHILES-CHROMOPHOES-ERYTHROCYTES)*			NO. OF MALES (AVERAGE COUNT)
	Nonpregnant	Pregnant	Postpregnant	
April.....	3 (24-14-22-6)			2 (40-7-24-0)
May.....	11 (30-8-18-6)	2 (24-14-23-9)		4 (28-6-25-6)
June.....	4 (34-4-27-6)	8 (43-10-16-4)		4 (26-10-24-5)
July.....		10 (27-16-24-5)		3 (29-21-20-1)
Aug.....	2 (30-12-19-12)†	3 (33-14-21-6)	5 (28-15-21-6)	4 (30-23-32-2)
Sept.....	1 (21-19-26-4)†		8 (25-14-20-5)	5 (21-13-21-0)
Oct.....	1 (20-17-19-17)†		2 (28-10-23-5)	3 (33-7-24-2)
Nov.....	2 (23-16-16-9)†		1 (34-8-25-4)	3 (27-12-21-14)
Dec.....				
Jan.....			2 (26-12-19-14)	2 (28-16-21-10)
Feb.....			1 (26-14-20-21)	2 (28-12-21-15)
Mar.....			1 (17-12-17-12)	3 (25-9-24-8)
Total number.	24	23	20	35

* Numbers in parenthesis represent the order of cells and are the averages for the animals used that month.
† Cell counts in virgin females.

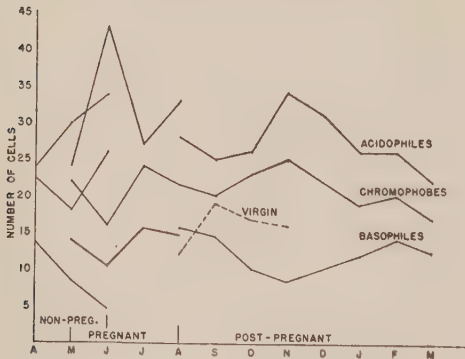
of cytoplasm and the fact that one-fourth of the cells have orange nuclei would indicate either transitional stages in light-blue basophile development or

mal peaks (Chart IV): one during pregnancy and the other during hibernation. Upon emergence from hibernation a two-thirds decrease in number (marked secretory activity of light basophiles) is undoubtedly associated with the release of gonadotropic substances, which become effective the middle of May, when climatic and food conditions improve, and which produce a tremendous growth of the largest group of ovocytes (Chart III).

During early pregnancy, when the number of basophiles is maximal, a temporary drop in number may be correlated with the growth and macroscopic appearance of a new third group of ovocytes. Otherwise, little growth is apparent in the remaining ovocytes during the period of gestation, when basophiles are most abundant.

In the postpregnant female, as basophiles decrease in number during September and October, there is continued slow growth of the three macroscopic groups of ovocytes into early hibernation, when the stage of ovocyte develop-

CHART IV
SEASONAL CELL CHANGES IN THE FEMALE
PITUITARY, 1941-42



secretory activity. Their increase in number and subsequent decrease may serve as a constant source of supply for the active, degranulating, and vacuolating light-blue basophiles which revert to chromophobes or degenerate.

The numbers of basophiles throughout the year reveal, as in the male, two maxi-

ment found in the emerging females is attained. Evidence of secretory activity of light basophiles is present on into fall, when the deep-blue basophiles increase; these predominate during early hibernation and give rise to the large light-blue basophiles that dominate in the spring.

Corpora lutea present in the ovary during pregnancy and slowly regressing in September and October bear a relationship to the pituitary (Clausen, 1940) and may account for the sex difference in basophile number, which is lower in the female. Mature virgin females possessed a larger number of basophiles than the pregnant females (Chart IV). Their number was more nearly like that found in the male. The number of basophiles remained high in fall, when those of the postpregnant female decreased.

DISCUSSION

A correlative study of the reproductive cycle in the male and female garter snake (*Thamnophis radix*) and of the pituitary gland necessitates the delineation of phases of the former and quantitative and qualitative alterations in the latter. Gonad activity of the snake living in a temperate climate is characterized by morphological and physiological changes during the spring, summer, and fall and an abeyance of activity in hibernation. In contrast to the single annual rhythm in this snake, reptiles in regions where climatic conditions are fairly constant may show repeated internal rhythms. Oviparous Javanese snakes lay eggs at intervals throughout the year (Kopstein, 1938), and the New Hebrides lizard (*Lygosoma*) breeds all year round (Baker, 1929). The annual fluctuation in metabolism in garter snakes that is associated with environmental change permits the single internal rhythm of gonadal activity to occur. However, these events in the two sexes

are not concurrent, as the male produces sperm the season prior to their use.

The testes of the garter snake undergo the most marked seasonal size changes in the summer, in this respect being like the turtle (Hansen, 1938; Risley, 1938). Lizards fall into two groups: those which attain maximal-sized testes in the spring, producing sperm for use in spring or early summer; and those which attain maximal-sized gonads in the fall, maintain during hibernation, and complete spermioteleosis in the spring (see Parts A and B in the section on the male for further discussion). The activity of the testes, which is most pronounced during the period of maximal size, shows the inception of spermatogenesis in May and probable secretory activity of the large interstitial cells, as evidenced by a smaller, denser nucleus and vacuolated cytoplasm. Similarly, when spermiogenesis is completed in the fall and the testes are regressing in size, interstitial cells attain their maximal size. This testicular activity during the spring and fall may be associated with the decreasing number of basophiles in the spring and fall.

The female possesses three groups of ovocytes. The largest group shows marked growth in May and early June but has a previous history of slow growth in former seasons. Upon ovulation and during subsequent gestation not only are corpora lutea present in the ovary but also the new third group of ovocytes appears in the ovary. Outside of the slight growth of this set of ovocytes to bring them into the macroscopic realm, there is little growth of the three groups during the remainder of the gestation period. Parturition in August is followed by regression of the corpora lutea and further growth of the three groups of ovocytes in the fall, when they attain the size seen in the spring. Even though the ovary shows most marked activity in the

spring, there is a slow growth present during early pregnancy and in the fall. As in the male, this gonadal activity may be associated with the two periods of basophile decrease.

Both sexes show gonadal activity from May until November. The marked changes in size concerned with the production of mature germ cells are not concurrent in the male and female. The production of sperm occurs after the female has ovulated, and under no circumstances could they be used in fertilization that season. The testes are minimal in size during hibernation and are not producing sperm. Sperm storage in the snake takes place in the ductus deferens, from where motile sperm can be secured over a period of 8 months. (In the turtle, sperm are stored in the epididymides, while lizards with maximal-sized testes in the fall do not complete spermiogenesis until spring.) Courtship and copulation may extend through the spring, when gonadal activity is in its incipient stages. Copulation may not involve insemination; nor is insemination immediately followed by fertilization, which occurs at ovulation the first part of June. Sperm storage occurs in the female from the time of insemination until fertilization. Consequently, the gestation period must be based on the time of ovulation and parturition data, rather than on mating dates. Difficulty in determining ovulation dates in a closely related species led the Blanchards (1941a) to speak of the "period of development" as "the time between the start in the egg of preparation for fertilization (which happens probably a month after mating) and the time of birth of young, without regard to either the point at which fertilization occurs or how long beforehand copulation takes place." In *T. sirtalis* the gestation period estimated from data presented by the

Blanchards is approximately 71 days. In *T. radix* the gestation period is approximately 9 weeks.

Physiological activity of the gonad hormones in the garter snake is concerned with the preparation of the reproductive tract for the reception of mature germ cells and a possible reciprocal action on the pituitary gland. That an estrogen is produced in snakes has been demonstrated by the assay of extracts of the ovaries of 324 specimens of *Bothrops jararaca* and *Crotalus terrificus terrificus* by Valle and Valle (1943). These authors state that Porto (1941) found progestational substances in the corpora lutea of Crotalidae. Fraenkel *et al.* (1940), in their studies on viviparous snakes, have shown that deluteinization of the ovary stopped development of embryos and that they were never born alive. Clausen (1940) found that ovariectomy in viviparous snakes (*Natrix*, *Thamnophis*, and *Storeria*) in early and middle gestation caused the resorption of yolk or the abortion of young, while in late gestation normal young were produced. Assay for androgen from the snake testes gave positive effects (Valle and Valle, 1943). The effect of the sex hormones in reptiles on the sex characters has been demonstrated by ablation and substitution experiments. They will not be considered here except to point out that normal quantitative relation of the hormone to the sex characters and to the pituitary gland or the excretion of the sex hormone is not known.

Sex hormones, administered to reptiles, may cause reduction in gonad size. Injections or the implantation of pellets of estrogens into male lizards (*Anolis*, *Eumeces*, and *Sceloporus*) produced a reduction in the size of the testes (see Poris, 1941; Forbes, 1941); into the female *Anolis*, no significant effect on the ovary. Testosterone pellets implanted

into male *Sceloporus* caused a reduction in testicular volume and a stimulation of spermatogenesis (Forbes, 1941), whereas injections did not affect the testes (Gorbman, 1939). Variable effects reported in reptiles are probably due to differences in dosage, mode of administration, and the time of administration. Moore, in the rat, interpreted reduction in gonadal activity following administration of sex hormones as being operative through the pituitary gland.

Cytological study of male and female *Anolis* pituitary glands following estrogen injection during the winter revealed decreased staining capacity and vacuolization of basophiles. This was interpreted as an indication of degranulation and increased secretory activity. Distention of blood sinusoids occurs, and the accumulation of colloid-like material is suggested as representing gonadotropic or thyrotropic hormone (Poris, 1941). The author also interprets vacuolization of basophiles as a degeneration change due to overstimulation. Severinghaus (1939) interprets the degranulating cells with enlarged Golgi apparatus and mitochondria as producing secretion. The exhaustion of the pituitary would lead to a decreased gonadotropic potency and cause partial involution of the gonad (see Fevold, 1939).

Castration in *Anolis* over a period of 3 months (February–April) and sacrifice at intervals of 3 weeks to 4½ months revealed no obvious histological changes in the pituitary, but the basophiles did increase slightly in number (Poris, 1941). In the experimental garter snake (castration at a low point in the normal basophile cycle and autopsy 124 days later) the number of basophiles increased to the same extent as the controls, which was a threefold increase from the initial level. The basophiles in the castrates, however, extended over a wider area.

Cytologically, there was no apparent difference; both showed numerous small deep-blue basophiles and relatively few light-blue basophiles.

Little is known regarding the effect of other endocrine glands on the pituitary gland in reptiles except for the work of Siler (1936), who thyroidectomized eight *T. radix* in the fall and eight in the spring. The fall animals were kept at 70° F. and were autopsied from 90 to 101 days later. The spring animals kept at room temperature, which varied with the outdoor temperature, were autopsied from 9 to 66 days later. Changes in the cells of the anterior lobe as a result of thyroidectomy as presented by Siler (1936) are:

(a) A transformation and enlargement of the chromophobe cells with an accumulation of basophilic granules to form, as a final stage, functional (?) beta cells. (b) Vacuolation of the beta cells with a loss of their fine granules to form a large non-granular cell, whose cytoplasm is chromophobic. (c) Degeneration of these transformed beta cells; the final form being a small cell with pycnotic nucleus and a very small rim of non-granular poorly staining cytoplasm. (d) A reduction in the number of alpha cells.

Similar changes were observed in the normal cyclical study of the pituitary gland, with normal fluctuation in temperature and thyroid function. Neither the sex of the animals nor the date of autopsy was given; nor was it stated whether gonadal function was initiated under winter laboratory conditions. In the lizard *Uromastix acanthimurus* an increase in the number of oxyphiles following thyroidectomy was reported by Viguier (1911). Changes due to thyroidectomy have been ascribed to all three types of cells by different investigators. Irrespective of the cell concerned, it is of interest to note here something of the mechanism of control of secretion, as determined by physiological studies of the potency of the pituitary, which

may have a parallel in gonadal-pituitary relations. No difference in physiological potency of normal, castrate, and thyroid-ectomized rat pituitary on the guinea-pig thyroid activation was observed (Hohlweg and Junkmann, 1933); but thyroxin-treated rat pituitaries had their thyrotropic effect decreased.

Abundant evidence is available for the control of testis function (Moore, 1939) and ovarian function (Van Dyke, 1936, 1939; Fevold, 1939; Smith, 1939) by two gonadotropic substances produced by the pituitary gland. A seasonal study of the cell changes in the pituitary gland of the snake, with a single annual reproductive cycle, offers certain advantages, which a short-cycle form like the rat does not offer, in that a given cytological condition may not be associated with the reproductive state at that time but the condition a day or two later. In the snake two types of acidophiles, which appear to arise independently from chromophobes, include large, coarsely granular, deep-red acidophiles and smaller, finely granular, pink-staining acidophiles. The former are present throughout the year in the acidophile area. They are abundant and more closely packed during the period of the animals' activity, whereas in hibernation chromophobe cells are present between them. In the spring and fall, cells with the granules dispersed have been interpreted as being in the process of degranulation.

The finely granular, pink-staining acidophiles appear in the chromophobe area and are apparently derived from those chromophobes, resembling them in size and shape. In the male they become fainter in the late summer and fall; in the female, in the fall. In both sexes they are chromophobic in the winter. Poris (1941) observed the pars anterior of *Anolis* as being deeply acidophilic in

late summer, fall, and winter and faintly acidophilic in the spring.

Cytological activity is evident in the basophiles, which are of two types; but they are believed to be phases in the activity of a single cell. Small, coarsely granular, deep-blue basophiles in the transition zone of the pars anterior are present in the summer, fall, and during hibernation. In the spring, upon emergence from hibernation, they practically all enlarge, degranulate, stain light blue (some nuclei stain orange and are irregular), vacuolate, disrupt, and become colorless, giving rise to small bodies with dense nuclei and a thin rim of irregular cytoplasm (summer). Whether these bodies degenerate or regenerate is not known. Large light-blue basophiles dominate in the spring, and, relative to the deep-blue basophiles, are fewer in number during the summer and fall in the female, not apparent in the male in fall, and practically absent in hibernation except for a few intermediate-sized light-blue cells.

Siler (1936) in *T. radix* and Hartmann (1944) in *T. sirtalis* recognized the types of acidophiles which have been considered here as two types, because of their difference in position and behavior. The basophiles have been considered in two categories, for convenience in relating their state to reproductive changes. Types of chromophiles in *Rana pipiens* and *Necturus maculosus* have also been described on the basis of granules and staining reactions (Zahl, 1937; Apling-ton, 1942).

The acidophiles have been associated with growth. Hereditary dwarfism in the mouse is associated with the absence of acidophiles, which can be restored with fresh anterior-lobe implants. Acromegaly and gigantism are associated with pituitary tumors, which are acidophilic. Pre-

dominantly acidophilic tissue of the ox pituitary has been shown to cause growth in tadpoles, whereas no such effect is observed when predominantly basophilic tissue is administered (Smith and Smith, 1923). The growth hormone from the ox pituitary has recently been isolated and found to cause a resumption of growth in hypophysectomized rats. In the dosage used, the preparation is free of other biologically active pituitary contaminants (Li and Evans, 1944).

The basophiles are generally considered in the literature to be the source of gonadotropic substance in the vertebrates (see Severinghaus, 1939, for review). Physiological investigation of the pituitary gland of reptiles concern effects of hypophysectomy, pituitary implantation, and gonadotropin injection.

Garter snakes, hypophysectomized for a short period when basophiles were increasing in number rapidly, showed a slight decrease in the weight of the testis and a sloughing-off of the germinal epithelium, indicating that the pituitary gland was actively producing a gonadotropic substance at this time. Longer periods (16, 44, and 66 days) produced a further regression of testicular size and a loss of testicular activity. Ten pituitary gland homeo-implants from July males into hypophysectomized males not only maintained normal spermatogenesis during a period of 9 days but also stimulated a 105 per cent increase in testicular weight. Schaefer (1933) hypophysectomized male garter snakes (*T. sirtalis* and *T. radix*) during the winter months and implanted a single hypophysis every other day for a period of 30 days; he reported the enlargement of tubules, the production of germ cells, and an increase in the size of the interstitial cells. The pituitary glands of winter animals under laboratory conditions may not be identi-

cal with those from animals in hibernation. However, these winter glands did produce stimulation, which indicates some storage of gonadotropic substance.

It is known that the pregnant female pituitary produces a gonadotropic substance which maintains the corpora lutea, for it has been shown that hypophysectomy, ovariectomy (Clausen, 1940), and deluteinization (Fraenkel *et al.*, 1940) stop development of young, producing abortion or resorption. Implantation of five homeoplastic hypophyses into a single snake (*Xenodon merremi*) induced ovulation in 6 days (Houssay, 1930); ovulation also occurred after five homeoplastic implants in *A. carolinensis* (Evans, 1935a).

Mammalian gonadotropins (Gonadin) injected into hypophysectomized garter snakes (during 16 days) maintained normal spermatogenesis, greatly stimulated the interstitial cells, and produced an 84 per cent increase in testis weight. Mammalian pituitary extracts failed to correct the increased respiratory quotient in the hypophysectomized snakes *Pituophis sayi sayi* and *Elaphe guttata* (Roth, 1942). In normal reptiles gonadotropins produce stimulation of the gonads of male *Eumeces laticeps* during the period of quiescence (Turner, 1935); of the male and female *A. carolinensis* (Evans, 1935a, b); of *Phrynosoma cornutum* (Mellish, 1936); and of immature male and female alligators (Forbes, 1937).

In the reptiles very few reports on cyclical changes in the pituitary gland are available. Altland (1939) found that only the female lizard (*Sceloporus u. undulatus*) had predominantly basophilic cords in May, when sexual activity was at its height. Poris (1941) reported an apparent increase in the number of faintly staining basophiles in *A. carolinensis* during the spring and early summer, when

gonads are most active. Hartmann (1944) found seasonal variation in the basophile number of *T. sirtalis* males.

A seasonal analysis of the basophile number in the male and female garter snakes (*T. radix*) shows two peaks—one during the period of maximal-sized testis and of pregnancy and the other during hibernation. They both have approximately the same seasonal onset and regression. The hibernation peaks are similar in numbers in the two sexes, and both show a predominance of small deep-blue basophiles and only a few intermediate-sized light-blue basophiles. This pituitary condition is interpreted as inactive in producing secretion; gonadal function is in abeyance during hibernation—in fact, all activities are at low ebb in hibernation. The peaks during the period of pregnancy and of maximal-sized testis differ in number in the two sexes. That in the male is higher than in the female and represents a sex difference. Even though the total number of basophiles is high and the majority of them are the small deep-blue basophiles, secretory activity is present during these periods in both sexes, as evidenced by the degranulation of the remaining light-blue basophiles.

Cytological evidence of the secreting basophile cells and evidence of the decrease in the number of basophiles, correlated with gonadal changes, are found in both sexes in the spring, when the ovaries show pronounced growth and testicular activity is initiated. Small deep-blue basophiles begin to increase and are most numerous during pregnancy and during maximal testis size. These are interpreted as a new supply of basophiles, which probably are not actively secreting but form a supply from which cells constantly pass into a secretory phase. That the male pituitary is

producing a gonadotropic substance even though the small deep-blue basophiles are increasing in number in July is shown (1) by the removal of the pituitary gland, which causes cessation of testicular activity, and (2) by the implantation of July pituitary glands, which maintains and stimulates testicular activity. That the pregnant female basophile cells are producing a gonadotropic substance in the first half of pregnancy, when their number fluctuates, is believed to be indicated by the growth of the new third group of oocytes.

In the female, following gestation, there is a slow decrease in basophile number as the supply of deep-blue basophiles is diminished by becoming large light-blue basophiles which degranulate. Corpora lutea during this period are regressing. The slow growth of the oocytes at this time indicates the production of a gonadotropic substance by the pituitary gland. This second regression in basophile number is not so striking as the pronounced degranulation of basophiles in the spring decrease in basophile number. The basophile number of the mature virgin female is higher than that of the pregnant female and more nearly like the number found in the male. The cells are predominantly small deep-blue basophiles and have been interpreted as being inactive in producing gonadotropic substance. Their number remains high even after the postpregnant female basophile number regresses. No apparent growth of the ovary was observed in the virgin females.

In the male the decrease in basophile number occurs after the first spermiogenic wave. That gonadotropic substance is produced is indicated by the increase in interstitial cell size.

In both sexes, in fall and during hibernation, small deep-blue basophiles in-

crease in number and predominate. A few intermediate-sized light-blue basophiles appear arrested in activity, as are all processes in the animal. Increasingly and decreasingly favorable climatic and food conditions in spring and fall, respectively, undoubtedly play a role in the degree of production of gonadotropic substance by the basophiles. During the optimal period of gonadal activity, gonadotropic substance is being produced. Sex hormones are similarly being produced and utilized by the reproductive tract. The degree of this utilization may supply the variable for reciprocal action on the control of the secretory activity of the basophiles.

SUMMARY AND CONCLUSIONS

1. The pars anterior shows zonation of three types of cells: acidophiles, basophiles, and chromophobes. Two types of acidophiles are described. Basophiles are grouped into light-blue and deep-blue cells, for convenience. The female has fewer basophiles during the reproductive peak than the male. This constitutes a sex difference. However, the basophile number of the mature virgin female is more nearly like that found in the male. The pituitary of the castrate male after 124 days shows a threefold increase in basophiles, which is slightly greater than the increase in the normal male.

2. There are seasonal changes in the pituitary of both sexes: (a) Coarse acidophiles are present throughout the year; they are abundant during the period of activity and scattered during hibernation. (b) Pink acidophiles in the chromophobe area in the male are present in the spring, becoming fainter in the summer and fall; they are present in the nonpregnant female in the spring and the pregnant female and absent in both sexes during hibernation. (c) Deep-blue basophiles are present in the summer, fall,

and during hibernation and in the spring give rise to the dominant large light-blue basophiles. (d) Light-blue basophiles, dominant in the spring, show extensive degranulation; they are relatively fewer during the summer and during hibernation.

3. In both sexes there are two peaks in the number of basophiles: (a) They have nearly the same seasonal onset and regression. Regression in the spring is correlated with marked gonadal activity; in the fall, with ovocyte growth and increase in interstitial cell size. (b) The hibernation peaks are similar and cannot be associated with gonadotropic activity. (c) The peaks during reproduction differ (sex difference), and secretory activity is present. (d) A slight fluctuation in basophile number during early pregnancy may be associated with the appearance of the new third group of ovocytes.

4. Animals hypophysectomized for a period of 9, 16, 44, and 66 days during the summer peak in basophile number showed a progressive atrophy and aplasia of the testis. July pituitary homeoimplants produced a 105 per cent increase in testis weight over a period of 9 days in hypophysectomized snakes. A total of 350 I.U. of equine gonadotropin over a period of 16 days increased testis weight 84 per cent in hypophysectomized snakes. In both groups spermatogenesis and interstitial cells were stimulated.

5. The maximal volume of the testis is from 500 to 990 per cent greater than the minimal volume, and the maximal weight is 430 per cent greater. Spermatogenesis occurs as a complete wave during the late spring and summer, with the most pronounced activity during June and July, which is associated with the maximal weight and volume of the testis.

Spermiogenesis is pronounced in August, with a secondary wave in late October. Sperm are stored in the ductus deferens during hibernation and are utilized the following spring. Interstitial cells show seasonal changes in size, staining, and cytology.

6. Three macroscopic groups of ova-

cytes show differential growth and ovulate in succeeding seasons. Corpora lutea persist during a 9-week gestation period, regress after parturition in August, and leave corpus luteum scars. The average number of young in 33 females is 13. Embryonic development ceases under hibernating conditions.

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PLATE I

(Magnification, 42×)

ABBREVIATIONS

<i>P.I.</i> , pars intermedia	<i>I.C.</i> , infundibular cavity	<i>S</i> , "stalk" of pars anterior	<i>B</i> , transition area of basophiles
<i>P.N.</i> , pars nervosa	<i>E.C.</i> , epithelial cyst	<i>R</i> , area of red acidophiles	<i>C</i> , area of chromophobes
<i>P.T.</i> , pars terminalis	<i>Co.</i> , "colloid" of pars intermedia	<i>P</i> , area of pink acidophiles	

FIG. 1.—Sagittal section of a May pituitary gland, showing the acidophile, basophile, and chromophobe areas of the pars anterior, the digitate pars nervosa (*P.N.*), and investing pars intermedia (*P.I.*). The anterior portion of the pars anterior (*top*) is separated, owing to the asymmetry of the lobe. The edge of an epithelial cyst (*E.C.*) is visible. (No. 26B, female.)

FIG. 2.—Several sections parasagittally to Fig. 1, showing the further extent of the pink acidophile area, the "colloid" in the residual lumen, the infundibular cavity, and the varying lengths of radiating digitate processes of the pars nervosa.

FIG. 3.—Sagittal section of a January pituitary gland, showing a more extensive corded chromophobe area, owing to the absence of pink acidophiles, the dark masses of blood cells in the sinusoids, the narrow basophile area (*B*), and the vascular pars terminalis (*P.T.*). (No. 260B, female.)

FIG. 4.—Several sections parasagittally to Fig. 3, showing the attachment (*S*) of the chromophobe area (*C*) to the pars intermedia (*P.I.*). Note the "colloid" in the residual lumen. The vascularity of the chromophobe area extends onto the surface of the pars intermedia. Digitate processes are fewer in number.

PLATE II

(Scale: $\frac{3}{8}$ Inch Equals 8 μ)

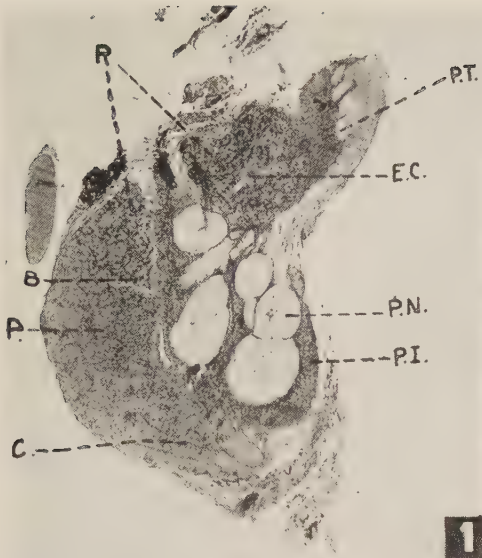
FIG. 5A.—Three red acidophiles (12 × 16 μ), showing the irregular cell shape, the indistinct cell membranes, and the dense granules, which appear coalesced, giving a finely "vacuolar" appearance to the cytoplasm. Some vacuoles are 1 μ in diameter.

FIG. 5B.—Three red acidophiles lining a

sinusoid, showing the large coarse granules scattered and packed in the cytoplasm.

FIG. 6.—Several pink acidophiles (8 × 8 μ), showing a regular, spherical shape with a ring of finely granular cytoplasm and a vesicular nucleus. These cells are present in the chromophobe area.

PLATE I



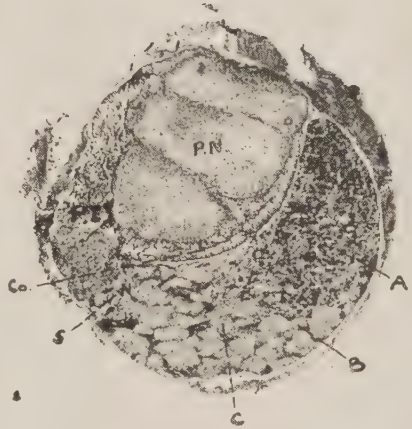
1



2

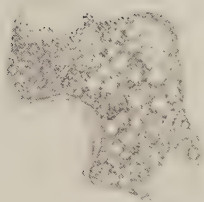


3



4

PLATE II



5A



5B



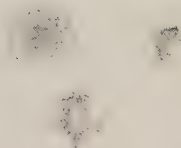
6



7A



8



7B



9

PLATE III

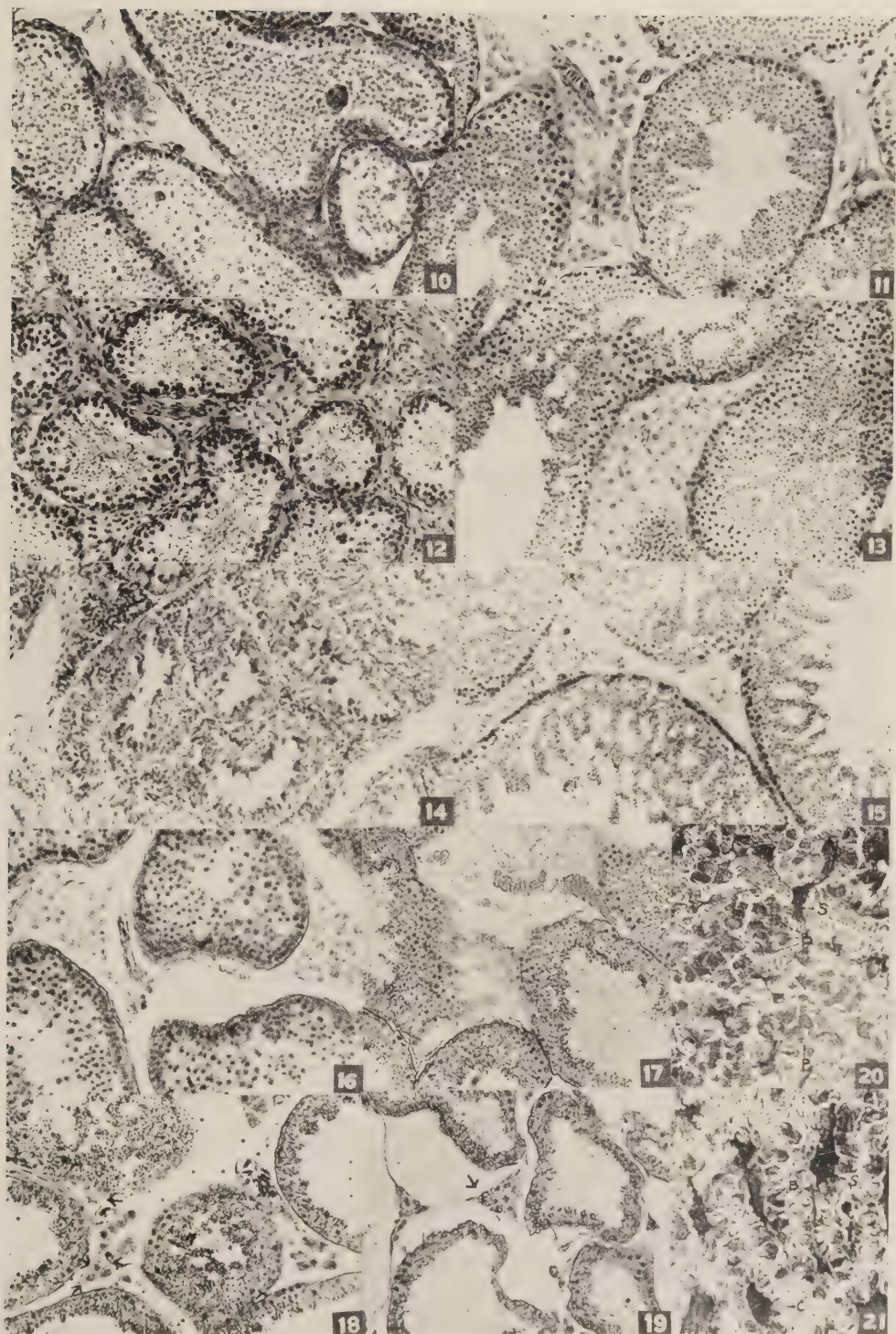


FIG. 7A.—Three small deep-blue basophiles ($6 \times 8 \mu$), showing the irregular shape of the cell. The dense granules appear coalesced, and one cell possesses a dense nucleus.

FIG. 7B.—Small vacuolated basophiles and basophile "bodies" with orange nuclei represent either degenerating or regenerating cells.

FIG. 8.—Several large ($8-14 \mu$) light-blue to colorless, basophiles, showing dispersed granules, vacuolization, pycnotic nuclei, and 6μ vesicular nuclei.

FIG. 9.—Chromophobes with little cytoplasm, 3μ vesicular nuclei, and indistinct cell membranes.

PLATE III

(Magnification: $150 \times$ for Figs. 10-19; $250 \times$ for Figs. 20-21)

FIG. 10.—Testis of a 9-day hypophysectomized snake, showing a narrow ring of germinal epithelium with sloughed cells in the lumen undergoing pycnosis and the small clumped interstitial cells. Control for Fig. 11. (No. 7F, 7-18-42.)

FIG. 11.—Testis of a 9-day hypophysectomized snake, maintained and stimulated by ten male pituitary homeo-implants. (No. 1F, 7-18-42.)

FIG. 12.—Testis of a 16-day hypophysectomized snake, showing shrunken seminiferous tubules consisting of a ring of spermatogonia and Sertoli cells, cellular debris in the lumen, and interstitial cells with irregular nuclei and vacuolated cytoplasm. Control for Fig. 13. (No. 8F, 7-25-42.)

FIG. 13.—Testis of a 16-day hypophysectomized snake which received a total of 350 I.U. of equine gonadotropin, showing marked stimulation of spermatogenesis and interstitial cells. (No. 11F, 7-25-42.)

FIG. 14.—Testis of a 66-day hypophysectomized snake, showing the completely disrupted germinal epithelium, the vacuolated, disintegrating cellular debris, and atrophy of the interstitial cells. (No. 23F, 9-4-42.)

FIG. 15.—Testis of normal September control, showing a second wave of spermiogenesis,

the large interstitial cells, and the abundant intertubular colloid. Cf. Fig. 14. (No. 36F, 9-4-42.)

FIG. 16.—Normal April testis, showing spermatogonia and primary spermatocytes, the final disintegration of left-over cells in the lumen, and the large interstitial cells. (No. 15.)

FIG. 17.—Normal August testis, showing marked spermiogenesis, the dispersed small interstitial cells, and the abundant intertubular fluid. (No. 53.)

FIG. 18.—Normal October testis regressing in size, showing the unevacuated cells and vacuolated interstitial cells (*arrow*). (No. 83.)

FIG. 19.—Normal December testis from hibernating snake, showing the collapsed seminiferous tubules with spent germinal epithelium, the large vacuolated interstitial cells (*arrow*), and the small interstitial cells with an irregular nucleus. (No. 93.)

FIG. 20.—Pituitary of a castrated male, showing numerous small deep-blue basophiles (*B*) extending over a wider area than in the normal control, Fig. 21. Few pink acidophiles (*P*). (No. 27F, 9-4-42.)

FIG. 21.—Pituitary of a normal male, showing small deep-blue basophiles lining the sinusoids (*S*) and numerous chromophobes (*C*). (No. 36F, 9-4-42.)

